



A First-Trimester Biomarker Panel for Predicting the Development of Gestational Diabetes

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Abstract

Objective: Serum markers measured early in pregnancy have been associated with the later diagnosis of gestational diabetes mellitus (GDM). This study aims to explore the performance of a panel of first-trimester biochemical markers for the prediction of GDM. **Methods:** A case-control study was performed that included 12 women who developed GDM and 60 controls matched for maternal and gestational age at blood collection. Levels of pregnancy-associated plasma protein A (PAPP-A), soluble endoglin, pregnancy protein 13, and adiponectin (Adipo) were measured on residual sera used in first-trimester screening for Down syndrome. Data were analyzed by nonparametric methods. A receiver operating characteristic curve was used to calculate the detection rate (DR) obtained with a panel of significant predictors for GDM. **Results:** Multiples of the median values for Adipo and PAPP-A were significantly reduced in GDM cases versus matched controls. Combination of Adipo and PAPP-A yielded a DR of 63.6% at a false-positive rate of 10%. Addition of body mass index (BMI) to this panel increased DR to 72.7%. **Conclusion:** This study suggests that first-trimester screening with Adipo, PAPP-A, and BMI may effectively identify women at high risk for the development of GDM.

Keywords

gestational diabetes, PAPP-A, adiponectin, multivariable screening

Introduction

Gestational diabetes mellitus (GDM) is defined as a carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy.¹ The American College of Obstetricians and Gynecologists² and the American Diabetes Association³ recommend routine screening for GDM; however, there is no general consensus for the screening method. In the United States, the “2-step approach” is most widely used for identifying pregnant women with GDM. In this protocol, the first step is screening at about 24 to 28 weeks’ gestation with an oral glucose challenge test (OGCT) using a 50-g glucose solution. In a systematic review of cohort studies of screening tests for GDM, the performance of OGCT at the 140 mg/dL (7.8 mmol/L) threshold yielded a detection rate (DR) and false-positive rate (FPR) of 70% to 88% and 11% to 31%, respectively.⁴ Screen-positive patients go on to the second step, where results of a 100-g 3-hour oral glucose tolerance test (OGTT) are considered diagnostic for GDM. In several European countries such as Italy, a “1-step approach” that omits the screening test (OGCT) is preferred. Results of a 75-g, 2-hour OGTT are considered diagnostic, but only women at high risk are offered testing. These include

women with body mass index (BMI) >25, previous GDM, maternal age >35 years, or residence in countries at high risk for GDM such as India, Pakistan, Bangladesh, the Caribbean, and the Middle East.⁵

The prevalence of GDM varies widely. In Northern Europe, low rates (0.6%-3.6%) are reported, while they are higher in Italy (6.3%)⁶ and the United States (7.0%).³ The International Association of Diabetes in Pregnancy Study Group reported that GDM prevalence ranged from 9% to 26% in 15 centers (United States, Singapore, United Kingdom, Thailand, Canada,

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Australia, Hong Kong, Barbados, and Israel) that participated in the Hyperglycemia and Adverse Pregnancy Outcome study.⁷

Gestational diabetes mellitus is a major cause of maternal and perinatal complications such as macrosomia, neonatal hypoglycemia, maternal hypertensive disorders, and increased rates of primary cesarean delivery. The main adverse impact of GDM on pregnancy is fetal macrosomia and the associated risk of shoulder dystocia.⁸ Women with GDM have higher rates of pregnancy-induced hypertension^{9,10} and are at higher risk of developing type 2 diabetes mellitus.¹¹

Demographic variables such as BMI, maternal age, and history are associated with the development of GDM; however, the performance of these measures as predictors is relatively weak with DRs of 32% to 58% at a 10% FPR.¹²⁻¹⁴ In a prospective cohort study, van Leeuwen and colleagues¹⁵ used demographic characteristics and medical history to predict 75% of GDM cases, but 43% of women were referred for glucose screening. A method that can more effectively select women at high risk of GDM would be beneficial, especially if it was implemented early in pregnancy. Early intervention could possibly avoid the development of GDM or avoid adverse maternal and fetal sequelae. To that end, we examined potential first-trimester serum markers for the prediction of the subsequent development of GDM.

Materials and Methods

Women being seen for care in the Division of Prenatal Medicine, Department of Medicine and Surgery (DIMEC), St. Orsola Hospital at the University of Bologna, Italy, a tertiary center for high-risk pregnancy, were recruited to have a 75-g OGTT, regardless of the risk factors for GDM. Recruitment was performed between May 2013 and December 2014. All women had singleton pregnancies and were euglycemic at the time of recruitment. Pregnancies with major fetal defects (congenital heart diseases and aneuploidies) or other known risk factors such as polycystic ovary syndrome, history of unexplained intrauterine fetal death, family history of diabetes mellitus, or a previous child with birth weight >4500 g were excluded. A possible selection bias could be represented by the Caucasian ethnicity and by the enrollment of a relatively high socioeconomic status (expressed as a combination of education, income, and occupation).

Among the 188 women initially recruited, 12 were diagnosed with GDM by OGTT at 24 to 29 weeks' gestation. A serum glucose level above ≥ 92 or ≥ 180 mg/dL at 1 hour or ≥ 153 mg/dL at 2 hours was considered diagnostic of GDM. Residual sera were collected from first-trimester combined screening for Down syndrome. Five samples from women without GDM ($n = 128$) were matched to each case for maternal age and gestational age (GA), for a total of 60 controls. All women gave informed consent to participate, and the study was approved by the local institutional review board (code CHD2014).

Immunoassay Testing

First-trimester serum PAPP-A was measured at the time of sample receipt using the B.R.A.H.M.S KRYPTOR automated immunofluorescent assay (Hennigsdorf, Germany; www.kryptor.net/). Values were converted to multiples of the median (MoM) based on GA, maternal weight, race, and smoking history.

Residual first-trimester serum samples from women who developed GDM and matched controls were retrieved from freezer storage and shipped on dry ice to Women and Infants Hospital, Providence, Rhode Island. All samples were coded so that assays were run without knowledge of group assignment. Total adiponectin (Adipo; R&D Systems, Minneapolis, Minnesota), soluble endoglin (sENG; R&D Systems), and human placental protein 13 (PP13; BioVendor, Czech Republic) were measured in singlicate by manual enzyme-linked immunosorbent assay methods. The PP13 had a sensitivity of 21 pg/mL, with an intra-assay coefficient of variation (CV) of 5% at 100 pg/mL. Adiponectin had a sensitivity of 390 ng/mL, with intra-assay CVs of 7%, 5%, and 10% at 2335, 8015, and 16 925 ng/mL, respectively. The sENG had a sensitivity of 0.16 ng/mL, with intra-assay CVs of 3%, 7%, and 9% at 0.58, 1.9, and 3.8 ng/mL, respectively.

Statistics

Data were matched for maternal age and GA in a 1:5 case-control study design. Median concentrations were calculated for each available marker in both case and control samples. The influence of BMI on marker levels was explored and adjusted as necessary. Regressions between BMI and markers were performed using only controls, excluding PAPP-A, which was already expressed as a MoM for clinical reporting. Nonparametric analyses (Mann-Whitney *U* test or Fisher exact test) were used to explore differences between cases and controls. Binary logistic regression was used to generate a posterior risk of GDM for each patient. Finally, the posterior risk generated by logistic regression was used in receiver operating characteristic curve analysis to calculate the combined DR, derived from the distributions of the most significant predictors of GDM. Differences were considered significant at a *P* value <.05.

Results

Table 1 shows the clinical characteristics of the women from whom serum was studied. Over short first-trimester interval studied, there was no effect of GA on serum marker levels. Therefore, only PAPP-A is expressed as a MoM with correction for GA and maternal weight at sample collection, since this was done for routine clinical reporting of combined test screening results. All markers were inversely correlated with BMI, but only PP13 reached statistical significance ($R^2 = 0.03$, F value = 5.45, $P < .02$, Figure 1). The PP13 values were therefore reported with adjustment for BMI.

Table 1. Demographic and Clinical Characteristics of Patients.^a

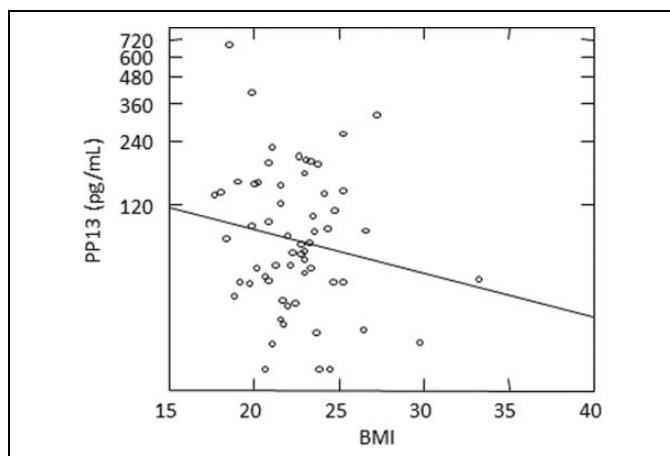
Variable	GDM, n = 12	Controls, n = 60	P Value
Gestational age at the time of blood draw (days)	86 (84-91)	86 (84-91)	1.000
Maternal age (years)	33.5 (24-40)	32 (24-43)	.084
Maternal age \geq 35 years (%)	41.6	21.6	.160
BMI	24.95 (19.5-33.1)	22.40 (17.7-33.3)	.001
BMI >25 (%)	13	60	.009
BMI >30 (%)	1.7	8.3	.221
Primiparous (%)	50	60	.539
Smokers (%)	16.3	3.3	.127
Prior GDM (%)	0	0	—
Gestational age at delivery (weeks)	37 (35-40)	39 (37-41)	.003
Vaginal delivery (%)	58.3	88.3	.023
Neonatal weight (g)	2950 (2290-3830)	3310 (2380-4080)	.015
35-37 weeks ^b	2662 (2290-2980)	2615 (2570-2660)	1.000
38-41 weeks ^c	3295 (2920-3830)	3345 (2380-4080)	.884

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus.

^aData are expressed as medians (range) or percentages.

^bSix cases and 2 controls.

^cSix cases and 58 controls.

**Figure 1.** Linear inverse correlation between pregnancy protein 13 (PP13) and body mass index (BMI) in controls. * $P < .05$.

Median first-trimester serum marker levels (interquartile range) are shown in Table 2. Figure 2 represents the box and whisker plots of the studied markers stratified by group. The levels of Adipo and PAPP-A MoM were significantly different between GDM cases and controls. On the other hand, levels of sENG and PP13 did not differ between GDM cases and controls. Analysis of PP13 stratified by BMI >25 and ≤ 25 did not change the results; there were no significant differences between GDM and control women for either BMI category (data not shown).

Table 3 reports the area under the curve and relative DR of each of the serum markers independently and in combination

Table 2. Median Value (IQR) for Biomarkers in Cases of GDM and Controls.

Variable	GDM (n = 12)	Controls (n = 60)	P Value
Adipo (ng/mL)	5189 (3789-7477)	9090 (6377-13543)	.012
Endoglin (ng/mL)	3.82 (3.30-5.17)	4.41 (4.41-5.48)	.661
PAPP-A (MoM)	0.70 (0.55-1.04)	1.10 (0.72-1.44)	.048
PP13 ^a (pg/mL)	62.43 (28.66-196.60)	80.13 (46.32-134.24)	.694

Abbreviations: Adipo, adiponectin; BMI, body mass index; GDM, gestational diabetes mellitus; IQR, interquartile range; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein A; PP13, pregnancy protein 13. ^aPP13 levels were adjusted for BMI.

for the detection of GDM. The BMI alone yielded a DR of 30%. As shown, PAPP-A MoM yielded a DR of 27.3%, followed by Adipo with a DR of 18.2%. The best performance was achieved using Adipo, PAPP-A MoM, and BMI, with 72.7% DR at a FPR of 10% (Figure 3).

Among the 12 cases of GDM, 8 women may have been considered high risk because of age >35 years and/or BMI >25 . Four women who developed GDM did not have these risk factors. Importantly, 2 of these 4 women without GDM risk factors were identified as high risk using the Adipo and PAPP-A serum marker screening test (Table 4).

Discussion

In this study, we have confirmed previous observations that first-trimester maternal serum levels of Adipo and PAPP-A are reduced in pregnant women who develop GDM. We found that Adipo levels in the first trimester were on average 43% lower in women who developed GDM versus euglycemic controls, similar to the magnitude of reduction reported previously by others.¹⁶⁻¹⁸ Our finding of a reduced first-trimester PAPP-A MoM (0.7 GDM/1.1 controls = 0.64 MoM) in women who developed GDM has also been corroborated by prior studies with larger sample groups (0.91 MoM in GDM¹⁹ and 0.69 MoM in GDM, adjusted for controls²⁰). To our knowledge, this is the first study to combine Adipo and PAPP-A at 12 to 13 weeks of gestation to predict GDM. We found that effective first-trimester screening for GDM can be provided using maternal BMI with serum Adipo and PAPP-A levels, achieving a 72% DR with a 10% FPR. Although our sample size is limited in this study, our multiple marker strategy has promise for the early detection of GDM and warrants further examination.

Prior studies have calculated the ability of serum markers to predict GDM. Adiponectin univariately detected 73% of cases but with a 33% FPR.¹⁶ The PAPP-A was similarly limited; first-trimester serum levels detected 81% of cases with 50% false positives.²⁰ While PP13 was significantly reduced at term in pregnancies with GDM versus controls,²¹ our study suggests that its value as a marker does not extend to the first trimester. Soluble endoglin did not differ in women with and without GDM at term²² or in the first trimester. Nevertheless, other

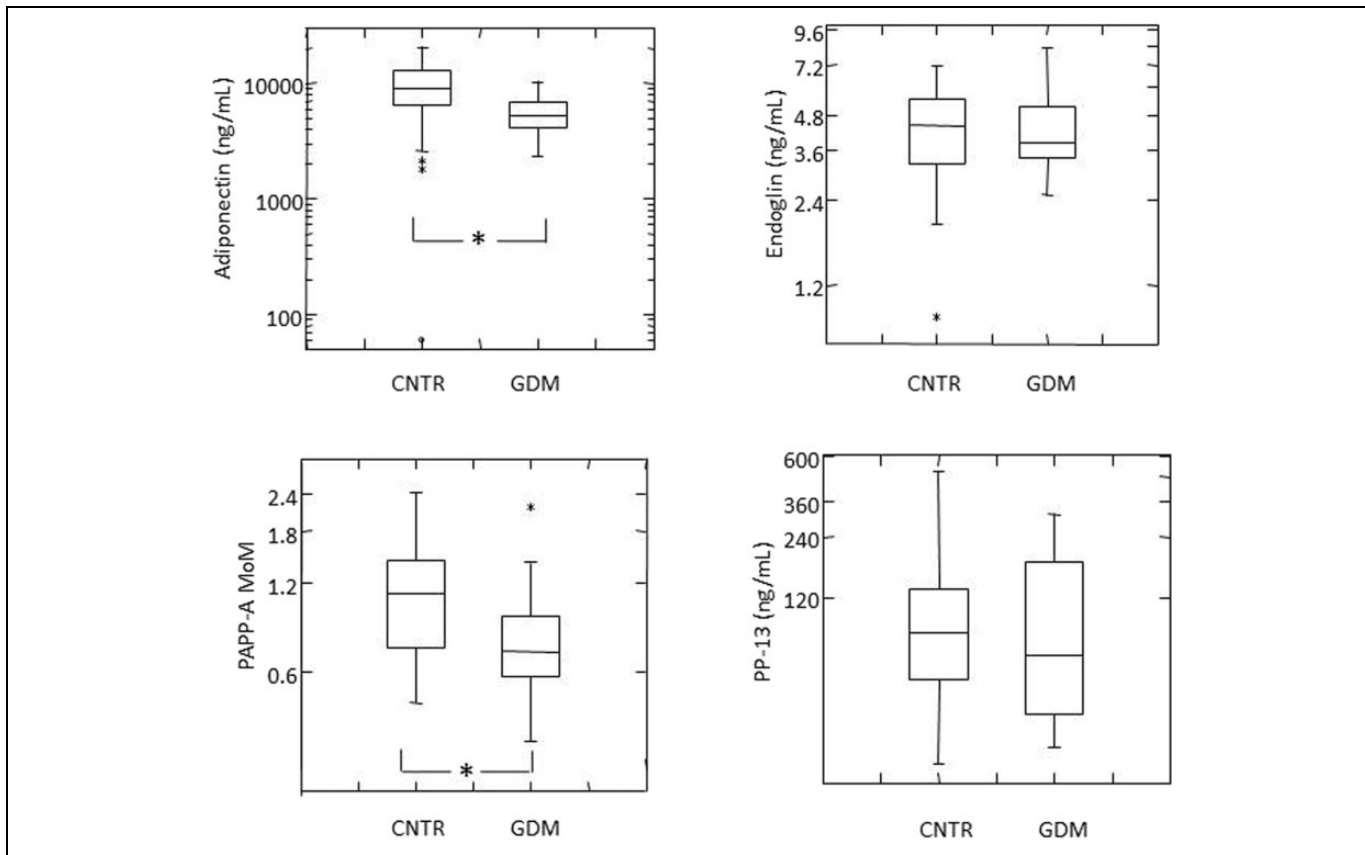


Figure 2. Box and whisker plot for the biochemical markers in controls (CNTR) and gestational diabetes mellitus (GDM) cases.

Table 3. Comparison of the Performance of Screening for GDM by BMI, Adipo, PAPP-A MoM, and Their Combination.^a

Variable	AUC	SE	DR	95% CI	p Value
Adipo	0.741	0.071	18.2	0.602-0.800	.012
BMI	0.810	0.072	33.3	0.669-0.950	.001
PAPP-A MoM	0.685	0.094	27.3	0.501-0.868	.048
Adipo + PAPP-A MoM	0.854	0.055	63.6	0.745-0.962	<.001
Adipo + PAPP-A MoM + BMI	0.877	0.045	72.7	0.789-0.964	<.001

Abbreviations: Adipo, adiponectin; AUC, area under the curve; BMI, body mass index; DR, detection rate; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein A; SE, standard error of the AUC.

^aDR is calculated at 10% false-positive rate.

promising candidates for first-trimester serum markers include placenta growth factor (PIGF) and sex hormone-binding globulin (SHBG), which were increased¹² and decreased,¹³ respectively, in women who developed GDM. Addition of 1 or both of these latter markers may further improve the current proposed panel.

There have been a few attempts to provide multiple marker screening for GDM, most utilizing demographic variables with serum, and none achieving the performance we observed for Adipo, PAPP-A, and BMI presently. For example, PIGF levels with maternal age and weight detected 71% of cases with 25%

FPR,¹² and SHBG in combination with several a priori risk factors led to 70% DR with 20% FPR.¹³ A multiple serum marker test of Adipo combined with SHBG and maternal risk factors had been one of the best models presented until now, with 78% DR and 20% FPR.¹³

There is a physiological rationale for the utility of Adipo and PAPP-A as markers of GDM. It is well known that gestational diabetes modifies trophoblast function, including cell proliferation, apoptosis, and cell cycle control. The GDM promotes an increased cellular proliferation rate²³ and, as a consequence, yields an increased placental mass and a higher rate of villous immaturity²⁴ in affected pregnancies. The PAPP-A is produced by trophoblast cells and modulates insulin-like growth factor 1 activity. It is postulated that reduced PAPP-A levels may drive hyperinsulinemia and insulin resistance.²⁵ Adiponectin, on the other hand, is derived from adipocytes and shown to promote insulin sensitivity. Levels of Adipo are reduced in nonpregnant women with type 2 diabetes mellitus, consistent with its putative role in GDM.²⁶

The method for implementing a routine first-trimester screening test for GDM must be carefully developed. In the present study, 9% of women routinely given OGTT at 24 to 29 weeks were diagnosed with GDM. It would be ideal if the first-trimester screen could avoid the need for such testing in low-risk women. It is also important to determine whether the first-trimester screen, in practice, would preferentially identify

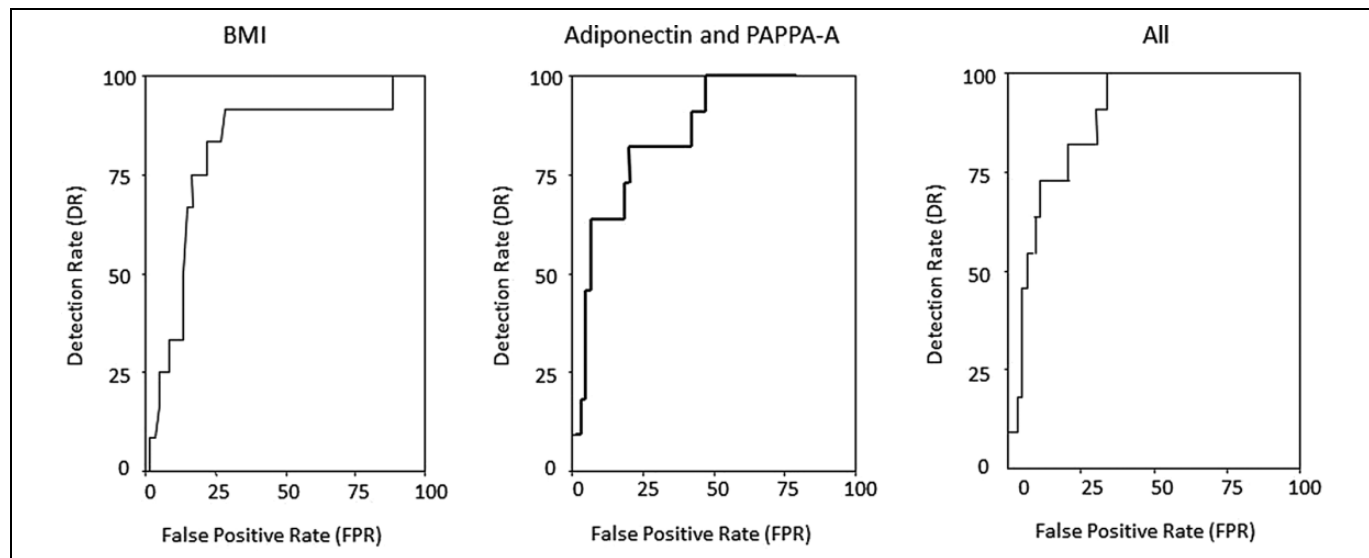


Figure 3. Receiver operating characteristic (ROC) curve analyses for body mass index (BMI), adiponectin (Adipo), and pregnancy-associated plasma protein A (PAPP-A) multiples of the median (MoM) for the prediction of gestational diabetes mellitus (GDM).

Table 4. Prediction of GDM According to Screening Method.^a

Case ID	Age >35	BMI >25	New Panel, Adipo+ PAPP-A MoM + BMI
Dietary treated			
1	–	+	+
2	+	+	+
3	–	+	+
4	–	–	–
5	+	+	+
6	+	–	–
Insulin treated			
7	+	–	–
8	–	–	–
9	–	–	+
10	+	+	+
11	–	–	+
12	–	+	+

Abbreviations: Adipo, adiponectin; BMI, body mass index; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein A

^aDetected cases are labeled with “+” and missed cases with “–.”

any subgroup of women with gestational diabetes, such as women who ultimately requiring insulin therapy. In the present study, there seemed not to be a bias in the detection of those women ultimately requiring insulin therapy; however, a much larger study is needed to address this. Other implementation issues must also be carefully examined for any new GDM screening program, such as acceptability, cost, and the reduction in adverse outcomes.

The value of first-trimester screening for the detection of GDM is to allow for early intervention. Lifestyle interventions such as modified dietary intake or physical activity increases may be effective in preventing or delaying GDM.²⁷ Alternatively probiotic therapy is being investigated as a means of prevention.²⁸

Conclusion

First-trimester screening with serum Adipo, PAPP-A, and BMI is a promising predictor of GDM. Additional markers, such as SHBG or PIGF, may provide enhanced performance. Early screening for GDM may be used to improve protocols for diagnostic testing at 24 to 28 weeks and may allow for early interventions to prevent or delay disease onset. This first-trimester screening strategy may reduce costs and improve maternal and fetal health outcomes.

Declaration of Conflicting Interests

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